

Biology of the Plant Cuticle

Edited by

MARKUS RIEDERER and CAROLINE MÜLLER

Julius-von-Sachs-Institut für Biowissenschaften

Universität Würzburg

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Biology of the Plant Cuticle

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Preface

During recent years the science of plant surfaces, and the cuticle in particular, has advanced at a fast pace, encompassing new fields of study along the way. Considerable progress has been made possible by the application of new concepts and techniques for investigating the biosynthesis, composition, structure and functional complexity of the plant cuticle.

We now have an increased understanding of the microscopic and sub-microscopic fine structure of the cuticular membrane as a whole, as well as of the cutin matrix and the associated wax deposits. By employing mutants and applying molecular biological techniques and advanced analytical tools, a much clearer image can now be drawn of the composition of cuticular waxes and the biosynthetic pathways leading to them. Intriguing variations can be found in the cuticular chemistry, morphology and function between and within plant species. Studies assessing the impact of UV radiation on plant life have emphasised the role of the cuticle and the underlying epidermis as optical filters for solar radiation. The field concerned with the diffusive transport of lipophilic organic non-electrolytes across the plant cuticle has reached a state of maturity, which makes it possible to quantitatively analyse and predict permeabilities based on physico-chemical predictors and to manipulate them *in vivo*. Recently, a new paradigm has been proposed for the diffusion of polar compounds and water across the cuticle. Within the context of plant ecophysiology, cuticular transpiration can now be considered in the perspective of whole-leaf water relations. New and unexpected roles have been assigned to the cuticle in plant development and in pollen–stigma interactions. Finally, much progress has been made in understanding the cuticle as a specific and extraordinary substrate for the interactions of the plant with microorganisms, fungi and insects.

Since the early 1970s, three books on the plant cuticle have been published. Only the first addressed all aspects of the subject; the other two were multi-author volumes arising from scientific meetings. Considering the progress made in this field, a book which deals comprehensively with plant surface characteristics and functions is overdue. The title, *Biology of the Plant Cuticle*, is intended to express the multidisciplinary and integrative approach to the subject. As functions are interconnected and rely heavily on the (bio)chemistry and properties of the cuticle, it is hoped that bringing together thus far disparate views of the subject will substantially advance the field of plant surface science. The book is also intended to provide a comprehensive overview and critical discussion of the current state of knowledge, paying close attention to the applied aspects of the field wherever appropriate.

Biology of the Plant Cuticle is aimed at a broad audience, ranging from biologists working on the molecular and whole-organism level to industrial agrochemists.

It is hoped that it will be of interest to phytochemists, plant (eco)physiologists, ecologists and environmental scientists, as well as to scientists and practitioners from the agricultural and horticultural sciences. In comparison with its predecessors, this book extensively considers the biological interactions occurring on plant surfaces and, therefore, is hoped to be of special appeal to scientists who, in the past, did not consider the plant surface *a priori* as a subject of prime importance. Thus, this volume is furthermore directed at phytopathologists, environmental microbiologists, entomologists and chemical ecologists.

The editors are indebted to the chapter authors for an enjoyable collaboration on this project and for timely delivery of carefully prepared manuscripts. In addition, the editors gratefully acknowledge the encouragement, advice and support continuously provided by Graeme MacKintosh and David McDade of Blackwell Publishing.

Markus Riederer
Caroline Müller

1 Introduction: biology of the plant cuticle

Markus Riederer

‘Does it make sense, and is it fun at all, to spend so much time with the outermost micrometer of a plant?’ This was the question a member of a search committee asked when the author applied for a job at a German university. As all scientists in this field know and deeply feel, it is fun indeed to study the plant cuticle and the plethora of processes related to it. The authors of this book hope that the reader will come to the conclusion that it is worthwhile to invest time, brains and funds into this endeavour.

The cuticle has often been called the ‘skin’ of the primary parts of higher plants, and in fact, the Latin word from which this term is derived (*cuticula*) means ‘thin skin’. The term cuticle has undergone a kind of evolution and profound changes in meaning during the last two centuries. At the beginning, the whole primary integument tissue or epidermis of a plant was called ‘cuticle’ stressing the convergence with animal skin, which is also cellular in nature. The modern usage of the word, meaning ‘a superficial film formed of the cutinized outer layers of the superficial walls of the epidermal cells’ (*Oxford English Dictionary Online*) of a plant, goes back to A.P. de Candolle. In 1827, he restricted the use of the French term ‘cuticule’ to the meaning in which it is used today (Wagenitz, 1996). Thus, the word cuticle is no longer used for a cellular layer but for a continuous extracellular membrane. In 1852, the word appeared in English for the first time in Henfrey’s translation of H. von Mohl’s ‘Grundzüge der Anatomie und Physiologie der vegetabilischen Zelle’ (OED Online). It is this term which will accompany us throughout this book.

1.1 The evolution of the plant cuticle

The cuticle as a structure has a very long history on the palaeobiological timescale. It is fortunate that the cuticle is a highly recalcitrant material which can easily resist decay for millions of years under favourable deposition conditions. It is fascinating that major chemical features like cutin composition are preserved over such prolonged periods of time (Ewbank *et al.*, 1996; Edwards *et al.*, 1997). Thus, essentially intact cuticles with clearly delineated epidermis cell silhouettes can be obtained from old sediments. The oldest remnants of plant cuticles date back to the boundary between the late Silurian and the early Devonian (about 400 million years ago) periods. The earliest cuticles, in the modern sense of the term which is assigned to higher plants, were found dispersed in sediments and belong to sporangia of rhyniophytoids. These specimens lack the impression of stomata while beginning with the

basal Devonian period, preserved cuticles show imprints from guard and accessory cells that are comparable to the modern stomatal apparatus. For a recent review on this subject see Edwards *et al.* (1996).

The finding that cuticles and stomata appear concomitantly in early kormophytes has profound impact on modern concepts of the evolution of vascular land plants and also on the interpretation of the selection pressure which acted on the evolution of stomata (Raven, 1977, 2002). Palaeoecophysiology has interpreted the simultaneous appearance of cuticles and stomata as evidence for the physiological adaptations to the colonisation of the land and thus for the relatively dry atmosphere by basal precursors of modern higher plants. Cuticles and stomata form a syndrome together with extended root systems, supracellular transport in vascular structures and the development of intercellular air spaces (Raven, 1977, 2002). These features are interpreted as necessary adaptations for photosynthesising homoiohydric life forms in an atmosphere with low water activity. The early cuticle probably also had additional functions equivalent to those of modern cuticles with defence against parasites, protection against ultraviolet (UV) radiation and water repellence being the most important ones.

1.2 Major functions of the plant cuticle

The cuticle is a structure that incorporates numerous functions of essential importance for plant life (Kerstiens, 1996b). This book treats the major functions in detail and, in most cases, devotes separate chapters to each of them. Nevertheless, a short synopsis is included in this introduction because it appears necessary to make one point very clear: the cuticle is a non-living though highly multifunctional structure into which numerous functions have been integrated. As will be shown later, this integration is sometimes not ideal as some physiological demands are in conflict with each other.

1.2.1 *Transpiration control*

As mentioned earlier, one of the major exigencies of the terrestrial lifestyle of higher plants is to have control over water relations. In order to stay alive, which essentially means to be more or less turgescient, the plant has to maintain the equilibrium between transpirational water loss and root water uptake. Any pronounced disequilibrium will severely compromise the viability and thus the fitness of the plant.

The control of transpiration from leaves, primary stems, flowers and fruits has two components: the stomata and the cuticle. Depending on the primary focus of scientific interest, the importance of either the stomata or the cuticle will be stressed by different authors. However, an effective control of transpiration is feasible only if the stomata *and* the cuticle act together in an optimised way. The low permeability of the cuticle makes it possible to control water loss by adjusting stomatal aperture.

But control will only work in a satisfactory way if the water loss across the cuticular surface is lower than the residual water loss through stomatal pores at optimal closure of stomata.

The cuticular permeability for water and transpiration confinement by the plant cuticle will be treated extensively in Chapters 8 and 9. This subject, of course, has met the interest of many researchers in the past. For general and early literature, the reader is referred to Stålfelt (1956), Schönherr (1982), and the textbooks by Larcher (2003) and Nobel (1991).

1.2.2 Control of loss and uptake of polar solutes

In principle, all organisms must have control over their inner milieu and therefore must have resistant integuments separating them from the environment. This is also true for terrestrial plants which would loose ions and polar organic solutes from the apoplastic solution unless they have a highly resistant cuticle that impedes the transport from the interior to the environment. Thus, the plant gains control over the loss of solutes and, at the same time, may modulate it by salt-excreting glands or hydathodes according to its specific needs. As the transport across the cuticle is symmetric, this membrane also hinders the uptake of polar substances from the outside. Control over solute loss and uptake is exerted by the same barrier properties of the cuticle as transpiration control. It might be speculated whether the need for controlling solute loss was an additional driving force in the evolution of the cuticular diffusion barrier.

A new view of the cuticular permeability of polar substances is currently evolving. There is increasing evidence that ions and small polar solutes move across the cuticle via continuous polar pathways that bypass the wax-based cuticular transport barrier. Chapter 8 (and partially also Chapter 9) will present this new view of polar solute (and water) transport across the cuticle and will put it in perspective with older work implying that such pathways may exist. For a thorough review of the older literature and for a primarily horticultural point of view on the subject of solute loss from plants (leaching), refer to the review by Tukey (1970).

1.2.3 Controlling the exchange of gases and vapours

When stomata are closed (which, on the average, is the case for approximately 12 h a day), the cuticle completely limits the loss and uptake of gases and vapours across the plant-atmosphere interface. This is true not only for water vapour as treated earlier but also for gases like carbon dioxide, oxygen, inorganic air pollutants and volatile organic compounds like terpenes (Lendzian and Kerstiens, 1991; Kerstiens *et al.*, 1992; Kerstiens, 1994). For highly lipophilic organic vapours, the cuticle is the preferred pathway of exchange even under conditions when the stomata are open (Riederer, 1995; Trapp, 1995). Exerting control over gas and vapour fluxes is, without any doubt, beneficial to the plant in most cases.

However, there is a conflict between controlling volatile exchange and photosynthesis. It has been shown experimentally in intact leaves with artificially clogged stomata that while the cuticle allows small amounts of carbon dioxide and water vapour to pass through, it markedly discriminates against the transport of carbon dioxide (Boyer *et al.*, 1997). A comparison with the properties of synthetic polymeric membranes like polyethylene, polycarbonate or polyester makes this property of the plant cuticle understandable. Woolley (1967) compared the permeabilities of plastic films to water and carbon dioxide and found that no synthetic material in existence has a higher permeability for carbon dioxide than for water. We can therefore conclude that intrinsic properties of a transport barrier against water and polar solutes confer low permeabilities to carbon dioxide and many other inorganic gases (Langowski, 2002). Evolution over the past 400 million years does not seem to have generated a membrane that can escape these physical constraints. This is the case even though a strong selective pressure acts towards a cuticle that allows photosynthesis during the light period but with the stomata closed.

1.2.4 Transport of lipophilic substances

The cuticle is the main aboveground interface for the exchange of lipophilic organic compounds between the environment and the interior of primary plant parts. All lipophilic compounds with low volatility or in solution have to cross the cuticle in order to enter or leave fruits, primary stems or leaves. The stomatal pathway is either not open to them (aqueous solutions of organic compounds) or is a very restricted route of exchange (semi-volatile compounds). The organic compounds in question may either be secondary metabolites of the plant or natural as well as anthropogenic compounds (pollutants, plant protection agents) occurring in the environment. From an applied point of view, the sorption and uptake of plant protection agents is of prime importance. Both the basic and applied aspects of this topic are discussed in Chapter 8. For publications covering the older literature, see these reviews and books: Van Overbeek (1956), Currier and Dybing (1959), Foy (1964), Sargent (1965), Bukovac (1976) and Hartley and Graham-Bryce (1980).

1.2.5 Water and particle repellence

After rains, many leaf surfaces are not covered by films of water and thus rapidly dry up. The cuticular surfaces of many plant species, at least their younger and pristine parts, are repellent to water and most water-based solutions. This is advantageous as water on the leaf surface may have several negative consequences for the plant; it (1) leads to leaching of ions and polar organic solutes from the plant's interior, and (2) creates suitable conditions for the colonisation by potentially harmful microbes like phytopathogenic bacteria or parasitic fungi. The latter aspect will be covered in Chapters 11 and 12 where the current knowledge on microbial communities and filamentous fungi on plant surfaces will be discussed in detail.

Certain plant surfaces may not only repel water and aqueous solutions but also microscopic particles like particulate aerosol, dust, spores and microbes. This is due to a self-cleaning mechanism based on the physico-chemical properties of some leaf surfaces and water droplets running off the surface taking along particles. This phenomenon has been termed Lotus effect and industrial applications have been explored (Barthlott and Neinhuis, 1997; Wagner *et al.*, 2003; Otten and Herminghaus, 2004). The fine structure of the cuticle and the chemical composition of cutin and cuticular waxes are covered in Chapters 2, 3 and 4, respectively.

1.2.6 Attenuation of photosynthetically active and UV radiation

One of the main driving forces for the colonisation of the terrestrial environment by plants is the luxuriant availability of radiation in the wavelength range from 400 to 800 nm in most cases. However, photosynthesis depends on a highly complicated and sensitive arrangement of pigments, proteins and membrane-enclosed compartments. This complex is easily damaged by excessive light. One of the protective mechanisms involves the cuticle: a dense cover of epicuticular wax crystals enhances scattering and reflection to a degree making tolerable the intensity of the radiation which reaches the photosynthetically active tissues in the interior of the leaf.

Another part of the electromagnetic spectrum hitting plant surfaces is UV radiation in the wavelength range from 280 to 400 nm. Excessive irradiation by UV results in damages in the photosynthetic apparatus and other vital parts of the plant cell. The cuticle, often together with the outer epidermal cell wall and the vacuoles of the epidermis, can contribute to an effective screening of UV radiation and thus to protecting the sensitive inner tissues. Chapter 6 covers the optical properties in the visible and UV range of the cuticle but also looks at properties of the epidermis and distinct sub-epidermal layers.

1.2.7 Mechanical containment

In a limited number of cases, the mechanical properties of plant cuticles support other structures like cell walls in maintaining the structural integrity of plant tissues. An economically important example for the mechanical importance of cuticles is fruit cracking in tomato and sweet cherry. In both cases, increasing internal pressure by uptake of water via roots or the fruit surface leads to the development of cracks. These cracks severely interfere with the economic and nutritional value of the fruits. Several studies have been performed on this issue either from an applied horticultural (Emmons and Scott, 1997; Bukovac *et al.*, 1999; Knoche *et al.*, 2002) or from a biomechanical (Wiedemann and Neinhuis, 1998; Matas *et al.*, 2004) point of view.

1.2.8 Separating agent in plant development

The cuticle plays a crucial role in plant development also and may be compared to a separating agent in developmental processes. Mutants with defective cuticles

exhibit increased water loss and, at the same time, extraordinary morphological abnormalities such as the fusion of organs. The emerging knowledge on the role played by the cuticle in cellular interactions and plant morphogenesis is extensively covered in Chapter 10.

1.2.9 Interface for biotic interactions

The cuticle-covered surface of higher plants is the main locality for major aboveground interactions with small organisms. On a microscopic scale, it is the interaction of bacteria, yeasts and fungi with the plant that may profoundly be influenced by cuticular properties. Features of the cuticle may have effects on adhesion, host recognition and mineral and carbon nutrition of the microbes as well as on the availability of liquid water. In addition, the cuticle may provide mechanical protection against the invasion by microbes. Cuticle-microbe interactions are treated in Chapters 11 and 12. For extensive reviews on these matters refer to Blakeman (1981, 1982, 1993) and Beattie and Lindow (1995).

On the macroscopic scale, the cuticle may interfere when insects or other arthropods interact with leaf surfaces. This may happen when a herbivore is searching for a suitable host for food or oviposition. Numerous cases have been reported where cuticular and leaf surface features in general influence herbivore behaviour and thus indirectly the integrity and fitness of the plant. This subject is reviewed in Chapter 13.

1.3 Convergence with other integuments

Not only plants but many other terrestrial organisms face at least some of the problems listed earlier. Very often, the main challenge is the danger of desiccation due to living in a dry atmosphere. The long-term maintenance of water balance must be solved by any terrestrial organism irrespective of its habitat. In order to hold back the water obtained from their surroundings, animals like plants typically possess an outer integument that greatly reduces the rate of water loss (Hadley, 1981, 1991).

In many species, the outer layers of the integument are covered and/or impregnated with more or less solid lipids which are primarily responsible for the observed waterproofing properties. This is especially true for plants and arthropods (insects and arachnids) both of which have a lipophilic matrix (cutin in plants, epicuticle in arthropods) with associated waxes. In both cases, these lipids are mixtures of long-chain aliphatic compounds as described for plants in detail in Chapters 3 and 4 and for insects in several reviews (Blomquist *et al.*, 1987; de Renobales *et al.*, 1991; Nelson and Blomquist, 1995). The cuticles of both arthropods and plants are continuous non-cellular membranes with multiple layers which cover the epidermis. In arthropods and in higher plants alike it is the physical structure, arrangement and composition of cuticular lipids that determines the waterproofing quality of the integument. Quantity and composition are species and age specific. For further

details on the convergence of plant and arthropod waterproofing properties and their relationship to chemical composition and physical structure of the cuticle, see the reviews by Hadley (1981, 1989, 1991).

This parallelism in the chemical, structural and physical properties of the outer layers of the integument is an outstanding example of convergent evolution in two widely divergent groups of organisms. The evolutionary success of wax-based transpiration barriers is extraordinary: if we take conservative species estimates as given by Wilson (1988, 1992), approximately 80% of all species on earth share a cuticle-like integument with low water permeability achieved by associated waxes. It may be speculated that two properties of mixtures of long-chain aliphatic molecules are responsible for this extraordinary evolutionary success: (1) waxes are plastic and can therefore follow growth and movements, and (2) they are multi-component, partly liquid or amorphous solids having self-healing properties which allow closing small defects inflicted on the integuments of plants or arthropods.

1.4 Objectives of this book

The science of the plant cuticle has received increasing attention among plant scientists as hitherto unknown functions and properties of this fraction of the epidermis have been discovered. Palaeobiologists, ecologists and especially plant evolutionary biologists become increasingly interested in cuticular remains and what can be deduced from their occurrence and structure. The volume of literature on the plant cuticle is growing at an increasing rate. A search in the BIOSIS database shows that during the last ten years (that is the time interval since the last book on the cuticle has appeared), approximately 2300 publications concerning the cuticles of plants have appeared.

The overall subject of this book has been treated in the past in several books. To the author's knowledge, the first modern experiment-based and comprehensive treatment of the cuticle and the associated waxes was provided by Frey-Wyssling (1938). Twenty years later, Martin and Juniper (1970) published a book which was the first to be exclusively devoted to the cuticles of plants. Thereafter, two volumes each compiling the proceedings of meetings devoted to the plant cuticle were edited by Cutler *et al.* (1982) and by Kerstiens (1996a), respectively. A small booklet on the surfaces of plants oriented at a general scientific audience was published by Juniper and Jeffree (1983).

Considering the progress made in this field since then, a new book covering the whole field of cuticular science appears overdue. Since Martin and Juniper (1970), the present book is the first written exclusively for this purpose and not derived from a scientific meeting. The title *Biology of the Plant Cuticle* has been chosen in order to express the multidisciplinary and integrative views of the subject. Cuticular functions are interrelated and heavily rely on the (bio)chemistry and the physical properties of the cuticle. Therefore, combining so far disparate views on this subject into a common perspective is expected to advance the field of plant surface

science substantially. Bringing the different functions together will delineate where and under which conditions they are in accordance and in conflict with each other depending on the special needs of the plant.

Obviously, the book is intended to provide a comprehensive and critical treatment of the current state of knowledge about plant surfaces and the cuticle in particular in its full depth and breadth. Recent developments having a pronounced impact on our understanding of the cuticle's fine structure, biosynthesis, composition, physical and transport properties are extensively reviewed in this book. For the first time, a comprehensive overview of cuticular functions in plant morphogenesis is given. Part of the book is devoted to the rapidly evolving field of biotic interactions taking place on plant surfaces. Where appropriate, special attention has been paid to the applied aspects of the field, especially in agricultural chemistry.

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